Environment, Health and Safety

SARS-CoV-2 Biological Agent Reference Sheet and Laboratory Guidance

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Check back periodically for updates as more information becomes available.

All labs working with the **SARS-CoV-2 virus**, **viral RNA**, or **samples suspected of containing the virus** must contact Cornell Biosafety and update their MUA with the Cornell IBC before starting any work. Risk group, biosafety level, and all other precautions noted here are subject to change after a risk assessment by Cornell Biosafety. While work with PCR products and molecular cloning is lower risk, residual nucleic acid on the researcher and through research space can confound surveillance results. Please see the guidance for labs performing these procedures.

All labs receiving infectious materials or nucleic acids capable of producing SARS-CoV-2 from outside the United States must apply to the CDC for an import permit. If the lab is receiving materials from another laboratory, the lab providing material may have a permit which also requires the recipient lab to have a permit.

Any labs generating **SARS-CoV/SARS-CoV-2 chimeric viruses by any deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids coding for SARS-CoV virulence factors** must notify EHS Biosafety as soon as possible. A November 2021 Interim Final Rule added this chimeric virus to the list of HHS select agents and toxins requiring registration with the Federal Select Agent Program.

Summary

Agent Type	Risk Group	Biosafety Level	Animal Housing Biosafety Level
Virus	RG-3	BSL-3	ABSL-3

Agent Characteristics

Description: SARS-CoV-2 may also be called 2019-nCoV, HCoV-19, and COVID-19. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a member of the *Coronaviridae* family and genus beta coronavirus that causes COVID-19

infections in humans. Coronaviruses are enveloped viruses that contain a single strand of positive-sense single-stranded RNA. SARS-CoV-2 is a newly emergent virus first reported as pneumonia of unknown etiology in Wuhan, China in late 2019. Viruses within this family that cause human disease include types 229E, NL63, OC43, and HKU1 which usually cause mild to moderate respiratory tract illness as well as SARS-CoV and MERS-CoV which can cause much more severe disease.

Host Range: humans, ferrets, cats, dogs, mink, and primates

Host Shedding:

- Direct contact
- Feces
- Saliva
- Respiratory droplets

Routes of Exposure to Humans: The primary transmission of SARS-CoV-2 is through close-contact, person-to-person spread. Transmission is driven by respiratory droplets. This mirrors what is known about similar coronaviruses, like SARS-CoV and MERS-CoV. Fomite transmission is a component in the spread of SARS-CoV-2: a person may sneeze or cough onto a surface leaving potentially infectious material. Subsequent contact with mucous membranes may lead to infection. Less clear is the role that airborne transmission may play. Currently, the reproduction number for SARS-CoV-2 infection (R_0≈2.5 for ancestral SARS-CoV-2, Delta variant R_0≈5.08, R_0 estimated to be 3.19 to 3.3 times higher for Omicron) is below the reproduction number for measles, known to spread via aerosols (R_0≈18).

- Aerosol/inhalation
- Direct contact
- Mucous membranes
- Contaminated items

Infectious Dose: estimations as low as 100 particles

Incubation Period: 2-14 days

Health Hazards

Signs and Symptoms: May appear between 2-14 days after exposure to the virus.

- Fever or chills
- Cough
- Shortness of breath or difficulty breathing
- Fatigue
- Muscle or body aches
- Headache
- New loss of taste or smell
- Sore throat
- Congestion or runny nose

- Nausea or vomiting
- Diarrhea

Immunizations: Available - three approved and authorized vaccines in the US

Prophylaxis: None available

Persons with pre-existing conditions such as moderate-to-severe asthma, hypertension, serious heart conditions, obesity, diabetes, chronic kidney disease treated with dialysis, liver disease, or are otherwise immunocompromised should consult with Cornell Health and avoid working with samples of SARS-CoV-2.

Agent Viability

Survival Outside Host	Disinfection	Inactivation
Up to 3 hours on printer paper or tissue paper Up to 2 days on wood or cloth Up to 4 days on glass and paper currency Up to 7 days on stainless steel or plastic and surgical masks	Products Registered in New York for Use Against COVID-19	All inactivation methods must be verified within your lab and approved by the IBC.

Several research studies have demonstrated interdependence between the survival of SARS-CoV-2, surface or material composition, temperature, and relative humidity. See the references for more information.

Laboratory Hazards

- High energy-creating activities (centrifugation, sonication, high-pressure systems, vortexing, tube cap popping)
- Splash/droplet-creating activities (shaking incubators, liquid culturing, mechanical pipetting)
- Equipment contamination
- Exposed skin/uncovered wounds

Laboratory Acquired Infections: 4 confirmed cases

Laboratory Handling Guidance

Risk assessment of lab activities is required before work begins including nucleic acid extractions. All labs working with RNA must

have an approved Memorandum of Understanding and Agreement with the IBC.

Biosafety Level	BSL-2	BSL-2 Enhanced	BSL-3
Materials and Activities	• Automated instruments and analyzers • Staining and microscopic analysis of fixed smears • Examination of bacterial cultures • Pathologic examination and processing of formalin-fixed or otherwise inactivated tissues • Molecular analysis of extracted nucleic acid preparations • Final packaging of specimens for transport • Using inactivated specimens	• Centrifugation, vortexing, or pipetting of viable clinical specimens collected from known or strongly suspected infected patients for research purposes (i.e urine, saliva, blood, NP swabs, OP swabs, etc) • Preparation of chemical- or heat-fixed specimens for microscopic analysis • Nucleic acid extraction of specimens for molecular analysis • Preparation of inactivated specimens for laboratory assessments	• Isolation and culture of SARS-CoV-2 virus • Amplification of the SARS-CoV-2 virus • Generation of infectious clones • Inactivation of materials with high viral load, including endotracheal (ET) aspirates, lung tissue, bronchoalveolar lavage (BAL) • Use of live SARS-CoV-2 virus in animal models • Use of live SARS-CoV-2 virus in functional models (Plaque forming unit assays, serologic virus capture/binding assays, etc.)

Labs handling clinical samples will establish a protocol requiring each researcher to take their temperature twice, daily, and log the information. If the researcher notices that their temperature exceeds 100.4°F, they will contact an appropriate health provider and leave the lab.

Inactivation

All labs working with the inactivated virus are expected to describe the inactivation protocol in their application to the IBC. All inactivation methods must be verified within the lab performing the method, after IBC approval. Data from the verification will be made available to EHS Biosafety and the Cornell IBC Chairs for review before work with inactivated materials may proceed.

Samples for RNA extraction are collected in storage buffers designed to inactivate proteins and preserve the RNA. Commonly these buffers often contain detergents, such as SDS, that should inactivate an enveloped virus. Data is still being developed and changes frequently. Use universal precautions for handling SARS-CoV-2 samples in storage buffers. However, inactivation by Trizol has been evaluated for the inactivation of MERS-CoV, SARS-CoV, and SARS-CoV-2. When the manufacturer's instructions are followed, there was no evidence of infectious virus when the results of that lysis were diluted to remove cytotoxic chemicals and added to growing cells. Labs will not be asked to validate this method.

Buffers AVL, ATL, and VXL (all from Qiagen) have all been tested with SARS-CoV-2. Both buffer ATL and VXL were capable of inactivating virus with and without BSA added to the solution. Buffer AVL required modification of the manufacturer's protocol. Labs using buffer AVL will be asked to validate this method.

Heat inactivation has been successful for various sample types that contain SARS-CoV-2. For example, BEI Resources distributes virus which has been inactivated using SARS-CoV-2 which has been inactivated by heating at 65°C for 30 minutes. Thirty minutes is conservative, based on prior research on the related virus, SARS-CoV, and has been adopted. Inactivation for an hour at 58°C has been shown to achieve the same results and is also acceptable.

Chemical fixation has focused on monolayers of cells and commonly use 10% neutral buffered formalin, 4% paraformaldehyde, and 1:1 methanol: acetone. Thirty minutes was sufficient to inactivate MERS, but little is known about SARS-CoV-2. The CDC Interim Guidance on Collection and Submission of Postmortem Specimens from Deceased Persons with Known or Suspected COVID-19 instructs that a tissue sample of 4-5 mm in thickness should be placed in at least 10 times the volume of the sample of 10% formalin and incubated for 72 hours for optimal fixation. Note: Paraffin-embedded samples will be fully heat-inactivated because the paraffin infiltration step places the sample at a temperature of 60-65°C for around 2 hours

All inactivation methods used need to be included in the submitted MUA and as written standard operating procedures (SOPs).

- Personnel must demonstrate proficiency in carrying out the procedure successfully and as written; this proficiency should be documented by the designated principal investigator.
    - If the lab wishes to use a technique other than the accepted methods described above, they must validate the method before use.
- Any inactivation procedure that requires opening the sample container or has the potential for aerosol creation should take place inside a biosafety cabinet.
- Before any changes to inactivation methods a new or updated SOP needs to be submitted to EHS Biosafety for risk assessment.

Training

For BSL-2 and BSL-2 Enhanced Work

- EHS Laboratory Safety Training (CULearn #2555)
- Lab-specific protocol training

For BSL-3

- BSL-3 training and demonstration of proficiency

Lab Engineering Controls

For BSL-2 and Enhanced BSL-2

- Class II Biosafety Cabinet (BSC)
- Centrifuge lids or safety cups or samples are loaded/unloaded inside the BSC

- Use of screw-top cap tubes in place of snap cap Eppendorf tubes to minimize aerosol generation and create better primary containment
- Aerosol resistant pipette tips

For more information about engineering controls in a BSL-3 lab, review the BSL-3 Program Manual.

Personal Protective Equipment

BSL-2 Lab where aerosol-generating activities can be contained within a BSC or other primary containment device

- Gloves (double gloves recommended)
- Front-close lab coat
- Safety glasses

BSL-2 Enhanced Conditions

- Double gloves. *Note: When coming out of a biosafety cabinet, outer gloves are disposed of in biohazard trash within the cabinet.*
- Closed-front lab coat
- Face Shield
- Respiratory protection, such as N95, is worn where aerosol-generating activities cannot be contained within a BSC or other primary containment device e.g. spill cleanup. Personnel wearing an N95 for these purposes must be part of the respiratory protection program.

In a BSL-3 Lab

- Powered Air-Purifying Respirator (PAPR)
- Disposable solid-front gown
- Lab-specific scrubs

**Please note: the IBC and EHS Biosafety & Biosecurity Group make final determinations of biosafety level and corresponding requirements after a formal proposal and risk assessment. For questions, please contact Cornell Biosafety.*

Waste Management: Regulated Medical Waste (RMW)

Shipping Guidance: review EHS Biological Substances Shipping

Animal Vivarium Guidance

Animal Housing Biosafety Level: ABSL-3

Animal Biosecurity: experimental animals are housed separately

Perform Inoculations: in Biosafety Cabinet (BSC)

Change Cages: in Biosafety Cabinet (BSC)

Exposure and Spill Procedures

Mucous Membranes: Flush eyes, mouth, or nose for 15 minutes at an eyewash station. See: Responding to Biological Exposures.

Other Exposures: Wash with soap and water for 15 minutes (open wounds, sores, etc.) or a minimum of 20 seconds for areas with intact skin.

Small Spills: Notify others working in the lab. Don appropriate PPE. For spills involving fecal material, cover the area of the spill with paper towels, work from the perimeter toward the center, use the paper towels to remove the spill and associated organic material. Discard contaminated paper towels. For spills involving fecal material and all other spills apply (or re-apply) 6% hydrogen peroxide on the spill site, Allow 20 minutes of contact time. After 20 minutes use paper towels to remove the 6% hydrogen peroxide. See: Responding to a Biological Spills.

Large Spills: Request assistance from the EHS Spill Team by calling CUPD dispatch. Call 911 from a campus phone or 607-255-1111 from a mobile phone.

Incident Reporting: Immediately report the incident to the supervisor and complete the EHS online injury/illness report as soon as possible.

Medical Follow-Up:

- For **students**, seek medical attention at Cornell Health or a local primary care provider. Call Cornell Health at 607-255-5155 (24-hour phone consultation line) or local urgent care.
- For **faculty and staff**, seek medical evaluation with a local primary care provider or urgent care. Cornell Health does not see employees for post-exposure care.
- *Emergencies*: Call 911 from a campus phone or 607-255-1111 from a mobile phone.

More Information

1. Biosafety in Microbiological and Biomedical Laboratories
2. COVID-19: Gastrointestinal Manifestations and Potential Fecal-Oral Transmission
3. Features, Evaluation and Treatment Coronavirus (COVID-19)
4. Genetic diversity and evolution of SARS-CoV-2
5. How COVID-19 Spreads | CDC
6. Products Registered in New York for Use Against COVID-19
7. List N: Disinfectants for Use Against SARS-CoV-2

8. Laboratory diagnosis of emerging human coronavirus infections - the state of the art
9. SARS-CoV-2/COVID-19 Toolbox | ABSA
10. Stability of SARS-CoV-2 in different environmental conditions
11. Symptoms of Coronavirus | CDC
12. COVID-19 Information for Specific Groups of People
13. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS–coronavirus 2
14. Pastorino B, Touret F, Gilles M, Lamballerie X De, Charrel RN. Evaluation of heating and chemical protocols for inactivating SARS-CoV-2. bioRxiv. 2020; 0–8. doi:10.1101/2020.04.11.036855
15. Darnell MER, Subbarao K, Feinstone SM, Taylor DR. Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV. J Virol Methods. 2004;121: 85–91. doi:10.1016/j.jviromet.2004.06.006
16. Kumar M, Mazur S, Ork BL, Postnikova E, Hensley LE, Jahrling PB, et al. Inactivation and safety testing of Middle East Respiratory Syndrome Coronavirus. J Virol Methods. 2015. doi:10.1016/j.jviromet.2015.07.002
17. Klompas M, Baker MA, Rhee C. Airborne Transmission of SARS-CoV-2. JAMA. 2020 [cited 31 Jul 2020]. doi:10.1001/jama.2020.12458
18. Riddell, S., Goldie, S., Hill, A., Eagles, D. & Drew, T. W. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. *V irol. J.* **17**, 145 (2020). doi:10.1186/s12985-020-01418-7
19. Liu, Y., & Rocklöv, J. (2021). The reproductive number of the Delta variant of SARS-CoV-2 is far higher compared to the ancestral SARS-CoV-2 virus. *Journal of Travel Medicine*, *28*(7). https://doi.org/10.1093/JTM/TAAB124
20. Ito, K., Piantham, C., & Nishiura, H. (2021). Relative instantaneous reproduction number of Omicron SARS-CoV-2 variant with respect to the Delta variant in Denmark. *Journal of Medical Virology*. https://doi.org/10.1002/JMV.27560
21. Nishiura, H., Ito, K., Anzai, A., Kobayashi, T., Piantham, C., & Rodríguez-Morales, A. J. (2021). Relative Reproduction Number of SARS-CoV-2 Omicron (B.1.1.529) Compared with Delta Variant in South Africa. *Journal of Clinical Medicine 2022, Vol. 11, Page 30*, *11*(1), 30. https://doi.org/10.3390/JCM11010030
22. Karimzadeh, S., Bhopal, R., & Huy, N. T. (2021). Review of infective dose, routes of transmission and outcome of COVID-19 caused by the SARS-COV-2: comparison with other respiratory viruses. *Epidemiology and Infection*, *149*. https://doi.org/10.1017/S0950268821000790

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